

5 WHAT IS CLAIMED IS:

1. A composition comprising at least two nanoparticle conjugates, each nanoparticle conjugate comprising:
 - a magnetic nanoparticle; and
 - at least one substrate moiety, in which each substrate moiety is linked to the nanoparticle and is chemically modified when the conjugate interacts with a target enzyme; wherein,
 - when the target enzyme is absent, the nanoparticle conjugates are essentially monodisperse in a liquid; and
 - when the target enzyme is present, the nanoparticle conjugates self-assemble into one or more nanoparticle conjugate clusters through the formation of intermolecular linkages between the chemically modified substrate moieties.
2. The composition of claim 1, wherein the the conjugates further comprise functional groups that link the nanoparticle to one or more substrate moieties.
3. The composition of claim 2, wherein the functional groups are selected from amino, -NHC(O)(CH₂)_nC(O)-, carboxy, or sulphydryl groups, wherein n is 0-100.
- 25 4. The composition of claim 1, wherein the magnetic nanoparticles each comprise a magnetic metal oxide.
5. The composition of claim 4, wherein the magnetic metal oxide is a superparamagnetic metal oxide.
- 30 6. The composition of claim 4, wherein the metal oxide is iron oxide.
- 35 7. The composition of claim 4, wherein the nanoparticles are an amino-derivatized cross-linked iron oxide nanoparticles.

- 5 8. The composition of claim 1, wherein the substrate moieties comprise a phenolic moiety.
- 10 9. The composition of claim 1, wherein the substrate moieties are chemically modified by oxidation.
- 15 10. The composition of claim 9, wherein the oxidation is a one electron oxidation.
- 20 11. The composition of claim 1, wherein the target enzyme is a protease.
- 25 12. The composition of claim 1, wherein the target enzyme is a peroxidase.
- 30 13. The composition of claim 12, wherein the peroxidase is myeloperoxidase.
- 35 14. The composition of claim 12, wherein the peroxidase is horseradish peroxidase.
- 40 15. The composition of claim 1, wherein each of the monodisperse nanoparticle conjugates has an average particle size of between about 40 nm and about 60 nm.
- 45 16. The composition of claim 1, wherein each of the monodisperse nanoparticle conjugates has an average particle size of about 50 nm.
- 50 17. The composition of claim 1, wherein each of the nanoparticle conjugate clusters has an average particle size of between about 400 nm and about 500 nm.
- 55 18. The composition of claim 1, wherein each of the nanoparticle conjugate clusters has an average particle size of about 450 nm.

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19. The composition of claim 14, wherein each of the monodisperse nanoparticle conjugates has an R1 relaxivity between about 5 and 30 mM⁻¹ sec⁻¹ and an R2 relaxivity between about 15 and 100 mM⁻¹ sec⁻¹.

10 20. The composition of claim 1, wherein the intermolecular linkages are covalent linkages.

21. The composition of claim 1, wherein the intermolecular linkages are non-covalent linkages.

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22. The composition of claim 1, wherein the formation of intermolecular linkages between the chemically modified substrate moieties is irreversible.

20 23. The composition of claim 1, wherein the formation of intermolecular linkages between the chemically modified substrate moieties results in crosslinking of the nanoparticle conjugates.

24. The composition of claim 1, wherein the composition further 25 comprises a fluid media.

25. The composition of claim 24, wherein self-assembly of the nanoparticle conjugates results in the spin-spin relaxation time of the fluid being decreased relative to the spin-spin relaxation time of the fluid having essentially 30 only monodisperse nanoparticle conjugates present.

26. The composition of claim 24, wherein the decrease in spin-spin relaxation time is dependent upon the concentration of the target enzyme.

35 27. The composition of claim 1, wherein the nanoparticle conjugate has a formula

X-(L)x-A, wherein:

X is a magnetic nanoparticle;

5 L is -NH-, -NHC(O)(CH₂)_nC(O)-, -C(O)O-, or -SS-, wherein n is
0-20;

10 A is substituted or unsubstituted aryl, substituted or unsubstituted
heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted
heteroaralkyl, substituted or unsubstituted aralkylamino, or substituted or
15 unsubstituted heteroaralkylamino; wherein substitutents are selected from halo,
hydroxy, C₁-C₄ alkoxy, or C₁-C₄ alkyl; and

x is 0 or 1.

28. The composition of claim 27, wherein X is magnetic metal oxide.

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29. The composition of claim 28, wherein the metal oxide is iron
oxide.

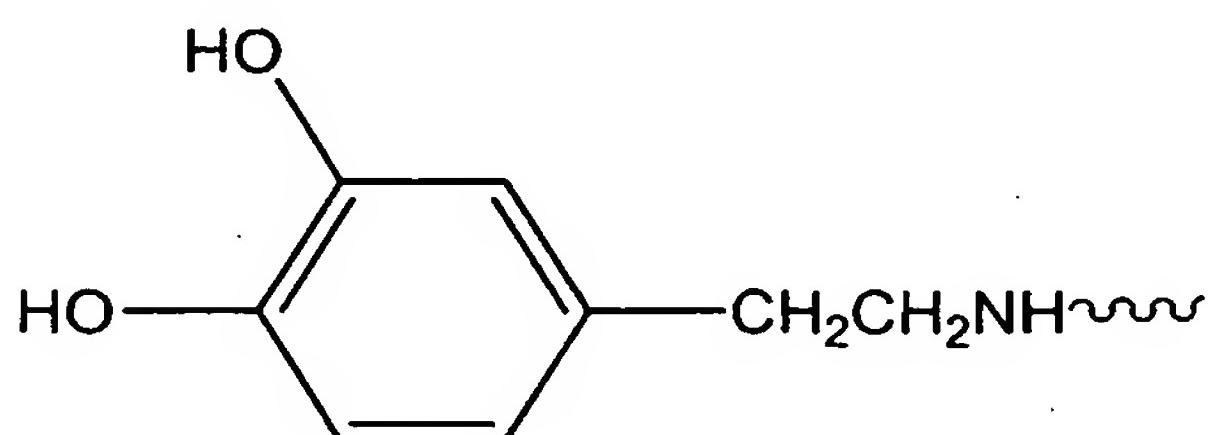
30. The composition of claim 27, wherein x is 1 and L is -
20 NHC(O)(CH₂)_nC(O)-.

31. The composition of claim 30, wherein n is 6.

32. The composition of claim 27, wherein A is substituted
25 aralkylamino, or substituted heteroaralkylamino.

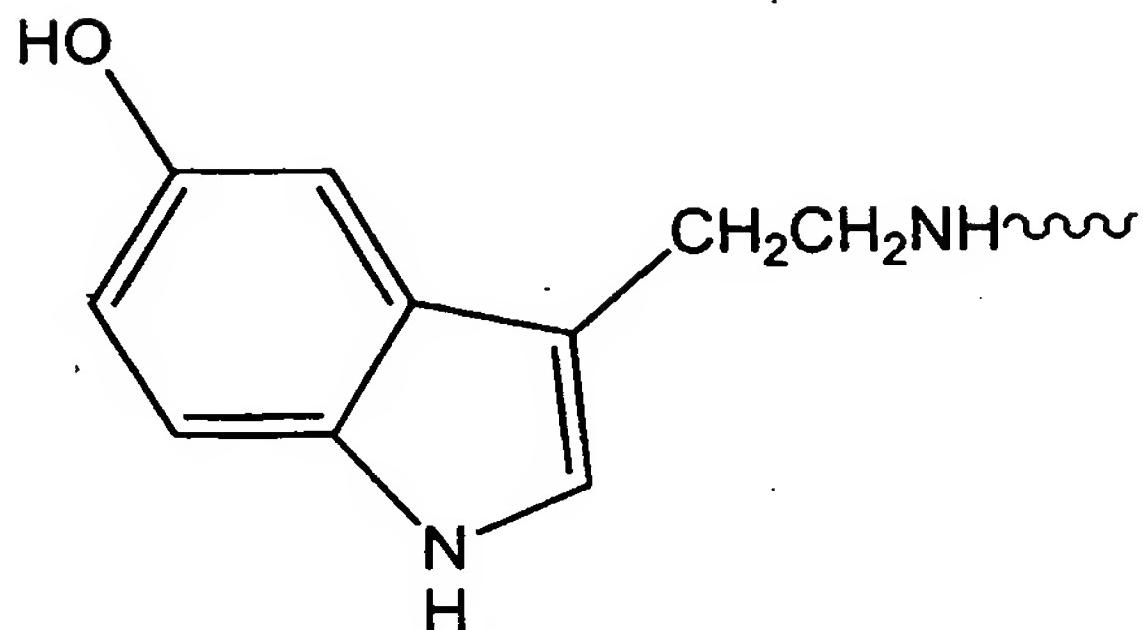
33. The composition of claim 32, wherein A is substituted with at
least one hydroxyl group.

30 34. The composition of claim 33, wherein A is:



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35. The composition of claim 33, wherein A is:



10 36. An *in vitro* method for detecting the presence of a target enzyme in a sample, the method comprising:

(i) providing a composition comprising at least two nanoparticle conjugates, each nanoparticle conjugate comprising a magnetic nanoparticle; and at least one substrate moiety, in which each substrate moiety is linked to the 15 nanoparticle and is chemically modified when the conjugate interacts with a target enzyme; wherein, when the target enzyme is absent, the nanoparticle conjugates are essentially monodisperse; and when the target enzyme is present, the nanoparticle conjugates self-assemble into one or more nanoparticle conjugate clusters through the formation of intermolecular linkages between the 20 chemically modified substrate moieties;

(ii) contacting the composition with a fluid sample;
(iii) allowing time (a) for the target enzyme to contact the nanoparticle conjugates and (b) for the nanoparticle conjugates to self-assemble 25 into clusters through the formation of intermolecular linkages between the chemically modified substrate moieties; and
(iv) determining the spin-spin relaxation time of the fluid over time,

wherein a decrease in spin-spin relaxation time indicates the presence of the target enzyme in the sample.

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37. The method of claim 37, further comprising the addition of hydrogen peroxide.

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38. The method of claim 36, further comprising the addition of glucose oxidase.
39. An *in vivo* method for detecting the presence of a target enzyme
10 in a subject, the method comprising:
- (i) administering to the subject a composition comprising at least two nanoparticle conjugates, each nanoparticle conjugate comprising a magnetic nanoparticle; and at least one substrate moiety, in which each substrate moiety is linked to the nanoparticle and is chemically modified when the conjugate interacts with a target enzyme; wherein, when the target enzyme is absent, the nanoparticle conjugates are essentially monodisperse; and when the target enzyme is present, the nanoparticle conjugates self-assemble into one or more nanoparticle conjugate clusters through the formation of intermolecular linkages between the chemically modified substrate moieties;
 - 15 (ii) allowing time (a) for the target enzyme to contact the nanoparticle conjugates and (b) for the nanoparticle conjugates to self-assemble into clusters through the formation of intermolecular linkages between the chemically modified substrate moieties; and
 - 20 (iii) determining the spin-spin relaxation time of the fluid over time,
- 25 wherein a decrease in spin-spin relaxation time indicates the presence of the target enzyme in the subject.
40. The method of claim 39, wherein the subject is a human.
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41. The method of claim 39, further comprising the step of identifying the subject as being in need of such detection.

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42. A self-assembling, nanoparticle conjugate comprising:
a magnetic nanoparticle; and
at least one substrate moiety, in which each substrate moiety is linked to
the nanoparticle and is chemically modified when the conjugate interacts with a
10 target enzyme; wherein,
when two or more nanoparticle conjugates are present and when the
target enzyme is absent, the nanoparticle conjugates are essentially
monodisperse in a liquid; and
when two or more nanoparticle conjugates are present and when the
15 target enzyme is present, the nanoparticle conjugates self-assemble into one or
more nanoparticle conjugate clusters through the formation of intermolecular
linkages between the chemically modified substrate moieties.

43. The nanoparticle conjugate of claim 42, wherein the conjugate
20 has a formula X-(L)^x-A,
wherein:
X is a magnetic nanoparticle;
L is -NH-, -NHC(O)(CH₂)_nC(O)-, -C(O)O-, or -SS-, wherein n is
0-20;
25 A is substituted or unsubstituted aryl, substituted or unsubstituted
heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted
heteroaralkyl, substituted or unsubstituted aralkylamino, or substituted or
unsubstituted heteroaralkylamino; wherein substitutents are selected from halo,
hydroxy, C₁-C₄ alkoxy, or C₁-C₄ alkyl; and
30 x is 0 or 1.

44. The conjugate of claim 43, wherein X is magnetic metal oxide.
45. The conjugate of claim 44, wherein the metal oxide is iron oxide.
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46. The conjugate of claim 43, wherein x is 1 and L is -
NHC(O)(CH₂)_nC(O)-.

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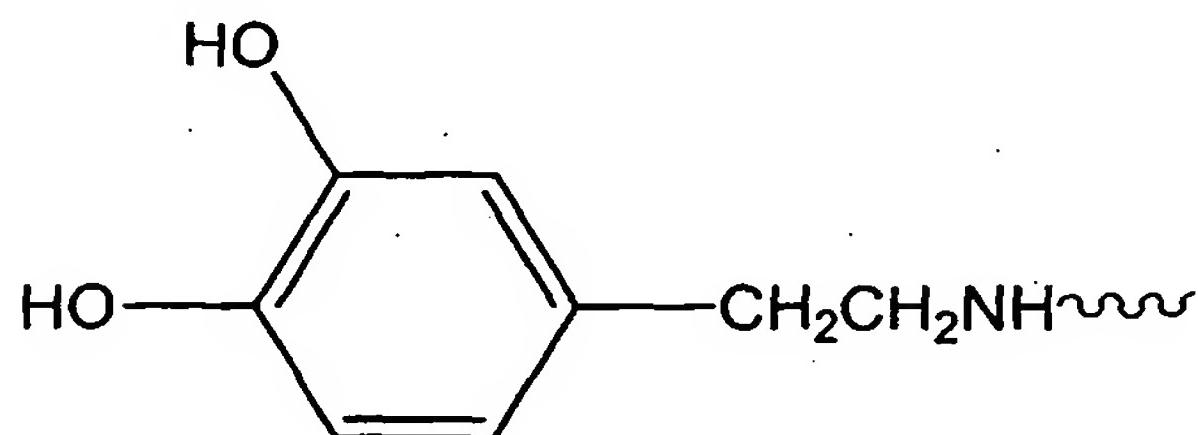
47. The conjugate of claim 46, wherein n is 6.

48. The conjugate of claim 43, wherein A is substituted aralkylamino, or substituted heteroaralkylamino.

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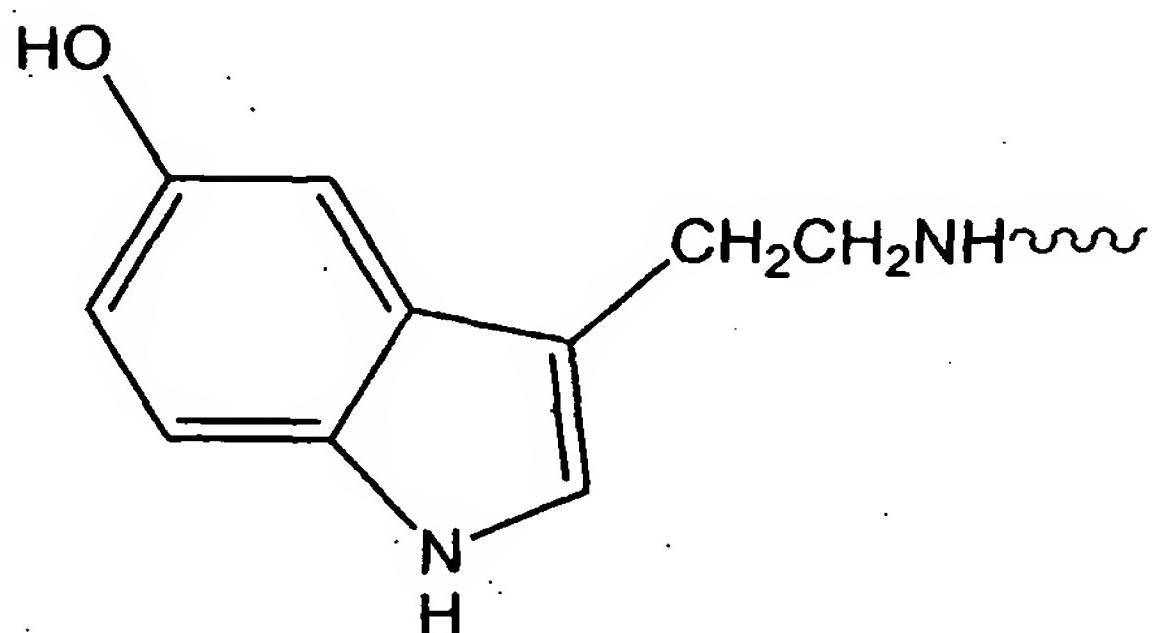
49. The conjugate of claim 48, wherein A is substituted with at least one hydroxyl group.

50. The composition of claim 49, wherein A is:



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51. The conjugate of claim 49, wherein A is:



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- 5 52. A packaged product comprising:
 a composition comprising at least two nanoparticle conjugates, each
 nanoparticle conjugate comprising:
 a magnetic nanoparticle; and
 at least one substrate moiety, in which each substrate moiety is linked to
10 the nanoparticle and is chemically modified when the conjugate interacts with a
 target enzyme; wherein,
 when the target enzyme is absent, the nanoparticle conjugates are
 essentially monodisperse in a liquid; and
 when the target enzyme is present, the nanoparticle conjugates self-
15 assemble into one or more nanoparticle conjugate clusters through the formation
 of intermolecular linkages between the chemically modified substrate moieties.